

U.S. PATENT APPLICATION OF SAMAL ET AL.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for generating five prime biased tandem tag libraries of cDNAs, comprising the steps of:
 - a) isolating a sample of mRNAs;
 - b) synthesizing double-stranded cDNAs from the mRNAs;
 - c) blunt-ending the double-stranded cDNAs;
 - d) attaching an adapter molecule to the blunt ends of the double stranded cDNAs to form a complex,
wherein the adapter molecule is a double stranded, synthetic oligonucleotide comprising:
 - 1) a recognition site for a type IIS restriction enzyme,
 - 2) a cloning site for releasing tags to a cloning vector, and
 - 3) a PCR primer site;
 - e) digesting the complex with a type IIS restriction enzyme to form released tags;
 - f) separating the released tags from the double-stranded cDNAs;

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g) amplifying the released tags to form amplified tags;

h) isolating the amplified tags;

i) concatenating the amplified tags to form concatenated tags;

j) amplifying the concatenated tags; and

k) isolating the concatenated tags.

2. The method of claim 1, wherein the type IIS restriction enzyme is selected from the group consisting of Ear I, Sap I, Alw I, Bmr I, Bsa I, BsmA I, BsmB I, Mly I, Ple I, Bbs I, BciV I, Fau I, Mnl I, Aar I, BfuA I, BspM I, Hph I, Mbo II, SspD5 I, Sth132 I, SfaN I, BseR I, BspCN I, Hga I, AceIII, Eci I, TaqII, Tth111II, Bbv I, RleAI, BcefI, Fok I, BceA I, BsmF I, StsI, Bce83I, BpmI, Bsg I, Eco57I, Eco57MI, and MmeI.

3. The method of claim 1, wherein the type IIS restriction enzyme is BpmI.

4. The method of claim 1, wherein the mRNAs are from a mammal.

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5. The method of claim 4, wherein the mRNAs are from a human.

6. The method of claim 1, wherein the released tags are comprised of 50 nucleotides or less.

7. The method of claim 1, wherein the released tags are comprised of 36 nucleotides or less.

8. The method of claim 1, wherein the released tags are comprised of 32 nucleotides or less.

9. The method of claim 1, wherein the released tags are comprised of at least 20 nucleotides.

10. The method of claim 1, further comprising sequencing the isolated concatenated tags to obtain a nucleotide sequence and comparing the nucleotide sequence to a known nucleotide sequence.

11. A method for generating five prime biased tandem tag libraries of cDNAs, comprising the steps of:

- d) isolating a sample of mRNAs;
- e) synthesizing double-stranded cDNAs from the mRNAs;
- f) blunt-ending the double-stranded cDNAs;

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d) attaching a first adapter molecule to the blunt ends of the double stranded cDNAs to form a first complex, wherein the first adapter molecule is a double stranded, synthetic oligonucleotide comprising:

1) a recognition site for a type IIS restriction enzyme,

2) a cloning site for releasing tags to a cloning vector, and

3) a PCR primer site;

e) digesting the first complex with a type IIS restriction enzyme to form first released tags;

f) separating the first released tags from the double-stranded cDNAs and attaching a second adapter molecule to the double-stranded cDNAs to form a second complex;

g) amplifying the first released tags to form first amplified tags;

h) isolating the first amplified tags;

i) concatenating the first amplified tags to form first concatenated tags;

j) amplifying the first concatenated tags;

k) isolating the first concatenated tags;

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- l) digesting the second complex with a type IIS restriction enzyme to form second released tags;
- m) separating the second released tags from the double-stranded cDNAs;
- n) amplifying the second released tags to form second amplified tags;
- o) isolating the second amplified tags;
- p) concatenating the second amplified tags to form second concatenated tags;
- q) amplifying the second concatenated tags; and
- r) isolating the second concatenated tags.

12. The method of claim 11, wherein the type IIS restriction enzyme is selected from the group consisting of Ear I, Sap I, Alw I, Bmr I, Bsa I, BsmA I, BsmB I, Mly I, Ple I, Bbs I, BciV I, Fau I, Mnl I, Aar I, BfuA I, BspM I, Hph I, Mbo II, SspD5 I, Sth132 I, SfaN I, BseR I, BspCN I, Hga I, AceIII, Eci I, TaqII, Tth111II, Bbv I, RleAI, BcefI, Fok I, BceA I, BsmF I, StsI, Bce83I, BpmI, Bsg I, Eco57I, Eco57MI, and MmeI.

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13. The method of claim 11, wherein the type IIS restriction enzyme is BpmI.

14. The method of claim 11, wherein the mRNAs are from a mammal.

15. The method of claim 14, wherein the mRNAs are from a human.

16. The method of claim 11, wherein the first or second released tags are comprised of 50 nucleotides or less.

17. The method of claim 11, wherein the first or second released tags are comprised of 36 nucleotides or less.

18. The method of claim 11, wherein the first or second released tags are comprised of 32 nucleotides or less.

19. The method of claim 11, wherein the first or second released tags are comprised of at least 20 nucleotides.

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20. The method of claim 11, further comprising sequencing the first and second isolated concatenated tags to obtain nucleotide sequences and comparing the nucleotide sequences to a known nucleotide sequence.

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